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Deciphering physio-biochemical, yield, and nutritional quality attributes of water-stressed radish (*Raphanus sativus* L.) plants grown from Zn-Lys primed seeds

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HIGHLIGHTS

- Water shortage appears to be expedited under the current worldwide climate change scenario.
- Zn is essentially required for the metabolism of proteins, carbohydrates, phosphates, auxins and nucleic acid synthesis.
- The nutrient solutions having amino acids present enhanced absorption, an effect due to their chelating property.
- Plants from Zn-Lys primed seeds exhibited improvement in biomass, photosynthetic pigments, yield and nutritional quality.
- The utilization of Zn-Lys maintained nutrient uptake and translocation by preventing drought-induced membrane lipid peroxidation.

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ABSTRACT

Water shortage appears to be expedited under the current climate change scenario worldwide. The present work was aimed to investigate the effects of zinc-chelated lysine (Zn-Lys) on germination and yield of water stressed radish plants. The research was comprised of two studies where the effect of Zn-Lys seed priming on germination attributes under PEG-induced water stress was investigated in the first experiment. In the second experiment, growth, physio-biochemical, and yield responses of water-stressed radish plants raised from Zn-Lys primed seeds were recorded. The seeds pre-conditioned with 0, 1.5, 3, 4.5, or 6 mg kg^{-1} of Zn-Lys was grown in petri-dishes and pots. Priming treatments significantly improved the germination attributes under water stress. Plants raised from primed seeds exhibited significant improvements in plant biomass production, leaf photosynthetic pigments, final root yield, and nutritional quality. Furthermore, the activities of superoxide dismutase (SOD) and peroxidase (POD) were increased, while the melondialdehyde (MDA) content decreased. Root flavonoids, ascorbic acid, carotenoids, protein, carbohydrates, fiber and lysine content were significantly improved due to Zn-Lys seed priming, both under water-stressed and non-stressed conditions. Moreover, plant's mineral nutrients such as K and Ca as well as Mg, Fe, P, and Zn of final harvested roots were also improved due to Zn-Lys seed priming. Overall, for the induction of drought tolerance and nutritional quality, Zn-Lys regimes of 3 and 4.5 mg kg^{-1} were most effective. It can be inferred that the Zn-Lys priming maintained a potential balance of nutrient uptake and translocation by preventing drought-induced lipid peroxidation of membranes.

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1. Introduction

The progression of global climate change and the challenge to sustainable agricultural production increases the need for effectively build resilience to water deficits (Arzani and Ashraf, 2016; Saha et al., 2016). The scarcity of water leads to stress development, which is now common across developing countries due to low rainfall and poor irrigation system/practices (Kang and Wan, 2005). According to recent studies, drought is the major factor resulting in reduced crop growth and yield (Noman et al., 2015; Ali et al., 2016). Plants are challenged to withstand abiotic stresses, and a close relationship exists between growth patterns

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coupled with physiological and biochemical processes that interfere with the plant growth, maturity, and production (Huang et al., 2007; Razmjoo et al., 2008; Leakey et al., 2009). Drought has pronounced effects on processes such as respiration, uptake of ions, translocation, carbohydrate breakdown as well as the mobilization and re-mobilization of nutrients.

Nutrients like calcium (Ca), potassium (K), and zinc (Zn) are necessary for normal plant growth as these act as enzymatic co-factors and are also involved in the stabilization of molecules, redox reactions, and the reduction of osmotic strain (Aery and Sarkar, 1991). Of these, Zn is considered to be the most important because of its involvement in metabolism of proteins, carbohydrates, phosphates, auxins, and nucleic acid biosynthesis (Kaya and Higgs, 2002; Zafar et al., 2016; Abdel Latef et al., 2017). A healthy plant maintains 27–150 mg of Zn per kg of biomass (Stevenson and Cole, 1999). However, most of the agricultural soils worldwide are deficient of Zn (Peck and McDonald, 2010) that cause abnormalities in plant growth such as chlorosis, sterility, small leaf and spikelet size (Cakmak, 2000).

Zinc can bind directly to metal transcription factor (TFs) and triggers its activation. Such an action is not known for other metal ions (Giedroc et al., 2001). It affects the uptake and translocation of water in plants (Kasim, 2007; Peck and McDonald, 2010). Due to its effectiveness in maintaining protein thiols, enzymes are less likely to be inactivated by reactive oxygen species (ROS) (Powell, 2000). Furthermore, it may act independently or as a co-factor of enzymes such as glutathione peroxidase and superoxide dismutase (Evans and Halliwell, 2001). Zinc also stabilizes the structure of various proteins such as Zn-finger transcription factors and metallothioneins. In a similar manner to copper (Cu), Zn is also a co-factor and component of Cu-Zn superoxide dismutase (Evans and Halliwell, 2001).

In plant nutrition, amino acids have an important role. The nutrient solutions supplemented with amino acids exhibit enhanced absorption, an effect arising from their chelating property and influence on the permeability of cell membranes (Rai, 2002). Among amino acids, lysine (Lys) acts as an important signaling amino acid stimulating cascades related to plant growth and plant responses to the environment. Methylated Lys is reported to be involved in vital processes such as flowering, development of seeds, gamete production, and fertilization in *Arabidopsis* (Yao and Shen, 2011).

Seed pre-conditioning (or priming) is a quick approach to modulate the effects of abiotic stresses on crop plants and has been reported to decrease seed dormancy, chances of desiccation, and promotes growth and flowering (Maroufi et al., 2011). Studies reveal that the seed priming with biostimulants is a best source to achieve optimum plant growth under harsh environmental conditions (Colla and Rouphael, 2015; du Jardin, 2015; Ali et al., 2017). Radish (*Raphanus sativus* L.), a member of family *Brassicacae*, is grown worldwide. The plant is a rich nutritional source and can be used in the treatment of various disorders, specifically liver diseases and jaundice (Vijayarengan, 2012). However, along with other crops under current climatic conditions, *R. sativus* production and nutritional quality is adversely affected by limited water supply. Review of literature reveals the direct influence of water availability on the growth, yield and quality of *R. sativus* (Kang and Wan, 2005; Hertel, 2012; Mahesh et al., 2013; Abdel, 2016). Documented evidence suggests that adequate water supply promotes the growth of *R. sativus* in comparison with water paucity (Mahesh et al., 2013; Abdel, 2016). Severe reduction in *R. sativus* yield was recorded under water deficit conditions (Abdel, 2015). Therefore, it was hypothesized that Zn-Lys seed priming might be helpful in the induction of drought tolerance, resulting in better yields and nutritional quality of *R. sativus*. The present study was conducted to evaluate the effects of Zn-Lys seed priming on seed germination attributes and growth in relation to oxidative defense mechanisms of *R. sativus* under conditions of water deficit. To date, the role of Lys based Zn chelation in abiotic stress tolerance for this crop has attracted little research. Furthermore, the study of the effects of Zn-Lys seed priming on root nutritional quality of water-stressed *R. sativus* plants was also within the scope of current research.

2. Materials and methods

2.1. Plant material

The seeds of *Raphanus sativus* (cv. mono-early) were obtained from Ayub Agriculture Research Institute, Faisalabad (AARI), Pakistan. The seeds were surface sterilized with 0.1% mercuric chloride for 3 min and washed thoroughly with sterilized double distilled water. The surface sterilized seeds were primed with Zn-Lys by using the concentrations of 0, 1.5, 3, 4.5 or 6 mg kg^{-1}, prepared by following the method reported by Leu (1991). Briefly, Zn-Lys chelation was prepared by dissolving 159.47 g of $ZnSO_4.7H_2O$ in 150 ml of water and adding an equimolar quantity (93.4 g) of L-lysine monohydrochloride. The solution was heated at 95 °C by constant stirring for 1 h. The mixture was dried in a microwave oven and used for the further experiments. The seeds were soaked for 12 h in solutions corresponding to 1.5, 3, 4.5 or 6 mg kg^{-1} Zn-Lys. The seeds primed with distilled water served as a control (0 mg kg^{-1}). The seeds were air dried and sown in petri-dishes (germination experiment) as well as in plastic pots (adult stage experiment).

2.2. Germination experiment

The germination experiment was executed in growth room under controlled conditions. The light duration was maintained for 10 h with a temperature of 25 °C±2, while the dark duration was 14 h with a temperature of 16 °C±2. The experiment was comprised of two sets of Petri-dishes (75 × 17 mm) supplied with double layer of filter paper. One set was taken as a control and supplied with half strength Hoagland's nutrient solution only and the other set of Petri-dishes was taken as drought stress, supplied with 8% PEG-8000, prepared in half strength Hoagland's nutrient solution having −0.46 Mpa (approximately 70% field capacity) osmotic potential. Each Petri plate was supplied with 10 ml of respective solutions and replicated thrice. The experiment was comprised of total 30 petri-dishes (15 in each set) and 15 seeds were sown in each Petri-dish. The counting of germinated seeds was done on daily basis, up till a constant count was obtained. Data was collected for the estimation of different germination attributes as given below;

2.2.1. Germination percentage (G%)

Counting of germinated seeds was done daily until a constant count was achieved. Percent seed germination was calculated with the help of following formula; Germination % age = Number of germinated seeds/Total number of seeds x 100.

2.2.2. Germination energy (GE)

Germination energy (GE) was calculated on the 4th day of sowing of seeds following (Ruan et al., 2002). Germination energy was calculated on the basis of percent germinated seeds at 4th day relative to the total number of tested seeds.

2.2.3. Emergence index (EI)

Emergence index (EI) was calculated by following the method of Association of Official Seed Analysis (1983). Emergence index (EI)

was calculated by using the following formula; EI = germinated seeds/Days of first count + … … … + germinated seeds/days of final count.

2.2.4. Mean emergence time (MET)

MET was calculated following Ellis and Roberts (1981) using the following equation;

$$MET = \Sigma\, Dn / \Sigma\, n$$

Where.

n = Number of seeds which were emerged on day D
D = Number of days counted from the emergence beginning

2.2.5. Days to 50% emergence (E_{50})

E_{50} was calculated by using the formula given by (Coolbear et al., 1984) with some modification.

$$E_{50} = t_i + (N/2\text{-}n_i)\,(t_j\text{-}t_i)/n_j\text{-}n_i$$

Where.

N= Final number of emerged seeds
n_i = Number of seeds emerged by count at time t_i when $n_i < N/2$
n_j = Number of seeds emerged by count at time t_j when $N/2 < n_j$

2.2.6. Co-efficient of uniformity of emergence (CUE)

Calculation of CUE was done by using the equation following (Bewley and Black, 1994).

$$CUE = \Sigma\, n / \Sigma\, [(t^{/} - t)^{2} \times n]$$

Where.

t = Time in days, beginning from day 0 or day of sowing
n = Number of seeds emerged on day t
$t^{/}$= MET

2.3. Pot experiment

2.3.1. Experimental soil and site

Experimental soil was collected from the agricultural fields (0–15 cm depth) of Government College, University, Faisalabad, Pakistan. It was thoroughly mixed, air dried, ground and passed through 8-mm sieve. The composite soil samples were again air dried, ground, and sieved through a 2-mm sieve. The physico-chemical properties of the soil were determined (Alloway, 1995) and are presented in Table 1. The experiment was carried out under open environmental conditions at the research area of Department of Botany Government College, University, Faisalabad located in the flat plains of northeast Punjab, Pakistan (31°24/N, 73°04/E).

2.4. Experimental arrangements

Thirty earthen pots (30 cm width x 60 cm depth) containing soil were used for growing plants. Half of the pots were given normal irrigation (100% field capacity) which was scheduled with 3-day interval while the remaining half was given drought treatment (70% field capacity). In order to maintain drought treatment i.e. 70% field capacity, soil moisture content of the pots was maintained on daily basis. The soil moisture contents were recorded as soil water tension by using Tensiometer (Irrometer, Model, LT-12 inch, Riverside, CA). To maintain the soil water content for required field capacity based on the tensiometric value, the change of 1 kpa in soil tension was equivalent to 1 mm of water. At the time of germination 1st irrigation was applied to all the pots and recommended NPK doses of fertilizers (0.03:0.025:0.025 g kg^{-1} soil) were applied in the form of urea, single super phosphate (SSP) and sulphate of potash (SOP), respectively. Before seed sowing, the soil was prepared and saturated with canal water. The seeds treated with different concentrations of Zn-Lys (as done in 1st experiment) were hand sown. Thinning was done 7 days after sowing (DAS), leaving four plants per pot. In order to keep the treatment uniformity, the uprooted seedlings were crushed and thoroughly mixed in the same pot. Mean daily day/night length was 14/10 h, mean maximum and minimum day/night temperatures were 25–15 ± 3 °C and 18–9 ± 2.5 °C, respectively and mean relative humidity (at daytime) was 50%. The water stress treatment was started after the 1st irrigation. The design of experimentation was completely randomized design (CRD) with three replicates for each treatment.

Table 1
Physical and nutritional properties of experimental soil.

Soil properties	
Soil Texture	Loam
Soil EC (d Sm^{-1})	4.1
Soil pH	7.4
Organic Matter (%)	1.242
Nitrate Nitrogen (mg Kg^{-1})	8.5
Available Potassium (mg Kg^{-1})	329.192
Available Phosphorus (mg Kg^{-1})	6.1
Total Magnesium (mg Kg^{-1})	70.1803
Total Calcium (mg Kg^{-1})	763.33
Total Iron (mg Kg^{-1})	143.872
Extractable Zinc (mg Kg^{-1})	3.91
Extractable Copper (mg Kg^{-1})	0.589
Manganese (mg Kg^{-1})	0.384
Molybdenum (mg Kg^{-1})	0.656
Boron (mg Kg^{-1})	60
Sulphur (mg Kg^{-1})	0.32

2.5. Estimation of morphological and growth attributes

After 20 days of water stress treatment, two plants per pot (per replicate) were uprooted and washed thoroughly. Subsequently shoots and roots were separated to record fresh weights as production of biomass. Afterwards, these were oven dried at 70 °C (for 72 h) for the evaluation of dry weights. The dry plants were then used for the estimation of different mineral nutrients. Shoot and main root lengths were also noted with the help of a meter rod. The remaining two plants were kept on growing in the prevailing growth conditions to obtain the final root yield and quality attributes.

2.6. Estimation of leaf relative membrane permeability (LRMP)

For the determination of leaf relative membrane permeability, 1 g leaves (ca 1 cm^2 each) were taken in test tubes containing 20 ml deionized distilled water (Yang et al., 1996). The contents were thoroughly mixed and the solution was assayed for initial electrical conductivity (EC0). Afterwards, the tubes were kept at 4 °C for 24 h and then again assayed for EC1. The test tubes containing samples were autoclaved at 120 °C for 30 min to determine EC2. Percent RMP was calculated as;

$$RMP\ (\%) = (EC1 - EC0\ /\ EC2 - EC0) \times 100$$

2.7. Leaf chlorophylls and carotenoid contents

The method of Arnon (1949) and Kirk and Allen (1965) was employed for the determination of leaf chlorophyll and carotenoid contents respectively by using 80% acetone as extraction solvent. The absorbance was read at 663, 645 and 480 nm by using a spectrophotometer (Hitachi U-2001, Tokyo, Japan) and specific contents were calculated by using specific formulas.

$$\text{Chl. } a = [12.7(\Delta A\ 663) - 2.69\ (\Delta A\ 645)] \times V/1000 \times W$$

$$\text{Chl. } b = [22.9\ (\Delta A\ 645) - 4.68\ (\Delta A\ 663)] \times V/1000 \times W$$

$$\text{Total Chl.} = [20.2\ (\Delta A\ 645) - 8.02\ (\Delta A\ 663)] \times V/1000 \times W$$

$$\text{Carotenoids (mg ml}^{-1}) = \text{A.car / Em 100\%} \times 100$$

Where,

Em (Emission) = Em 100% = 2500
A Carotenoid (μg/g FW) = ΔA480 + (0.114 × ΔA663) − (0.638 × ΔA645)
ΔA = Absorbance at respective wavelength
V = Volume of extract (ml)
W = Fresh weight of the sample (g)

2.8. Estimation of anti-oxidative enzyme activities in leaf and root

2.8.1. Extraction of antioxidant enzymes

For the extraction of antioxidant enzymes, 0.5 g plant material was homogenized in 10 ml of pre-chilled 50 mM phosphate buffer having pH 7.8. The plant material was homogenized using a pestle and mortar and the homogenized material was centrifuged at 20000 × *g* at 4 °C. The supernatant was separated and used for the estimation of total soluble proteins and the activities of antioxidant enzymes.

2.8.2. SOD and POD activity

The activity of SOD in leaf and root was measured by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm according to the method described by (Giannopolitis and Ries, 1977). The guaiacol oxidation method (Chance and Maehly, 1955) was used for the POD activity determination. The reaction mixture (3 ml) contained 0.1 ml enzyme extract, 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol and 40 mM H_2O_2. Changes in optical density of the reacting samples at 470 nm were recorded every 20 s.

2.9. Estimation of lipid peroxidation

Malondialdehyde (MDA) contents were determined by thiobarbituric acid (TBA) reaction following the method of Heath and Packer (1968) in order to assess lipid peroxidation level. Shoots and roots were homogenized (separately) with 0.1% trichloroacetic acid (TCA) using a POLYTRON ultra-mixer (PT 2000, Switzerland). The homogenates were centrifuged for 5 min at 10000 × *g*. The supernatant was added to TCA (20%) containing TBA (0.5%) and was heated at 95 °C for 30 min. The reaction was stopped by placing the tubes on ice and samples were centrifuged again at 10000 × *g* for 5 min. The absorbance of the supernatant was measured at 532 nm using Ultrospec 3000 (Biochrom Ltd. Cambridge, England) and adjusted for non-specific absorbance at 600 nm (absorption/extinction coefficient was 156 mM cm^{-1}).

2.10. Estimation of final root yield and root nutritional quality

After 40 days of the start of drought stress, the remaining two plants from each pot (per replicate) were harvested for the estimation of final root yield and its nutritional quality. After measuring the root fresh weight, diameter and final root length, half of the roots from each replicate were stored at −20 °C for the estimation of root quality attributes and the other half was oven dried for the estimation of varying nutritional attributes.

2.10.1. Estimation of root carotenoids

Extraction of carotenoids from fresh radish roots was done following Rodriguez et al. (1976). Five gram fresh root material was homogenized in 20 ml 80% acetone. The acetone layer was separated and mixed with 20 ml petroleum ether. For the separation of phases, water was added, and after the separation, petroleum-ether-carotenoid phase is made up to the volume of 50 ml. Petroleum ether extract was determined spectrophotometrically following the method of Ramos (1991) at the wavelength of 450 nm using the spectrophotometer (Hitachi, U-1800 Spectrophotometer, Japan) for the estimation of carotenoids. The concentration of carotenes expressed as β-carotene (g/100 ml) was calculated using the response factors as follows

$$\beta\text{-carotene} = A \times df \times V/E1\%\ 1\ cm \times w$$

where:

A − Absorbance
df − Dilution factor
E1% − Co-efficient of absorbency (2592 for petroleum-ether)
1 cm
w− Weight of sample (g)
V− Volume (ml)

2.10.2. Estimation of root ascorbic acid content

The content of ascorbic acid (AsA) in radish root was estimated following the method described by with (Mukherjee and Choudhuri, 1983) some modifications. The fresh plant material (5 g) was homogenized in 20% TCA. After filtration and centrifugation (10000 × *g*), one ml of the supernatant was reacted with 2 ml of 2% dinitrophenyl hydrazine solution (in acidic medium). Then one drop of ethanolic 10% thiourea solution was added to the mixture. The mixture was boiled for 20 min in a water bath. After cooling the mixture at 25 °C, 5 ml of 80% H_2SO_4 (v/v) were added to the mixture at 0 °C. The absorbance was read at 530 nm. The amount of AsA in the extracted leaf samples was worked out from a standard curve prepared using varying AsA standards.

2.10.3. Estimation of root flavonoid content

The total flavonoids in the roots were measured by the method of (Sultana et al., 2009). Briefly, 10 g fresh root was homogenized with methanol using an electric blender. The filtrate (1 ml) was diluted with 4 ml water in a 10 ml volumetric flask. Initially, 5% $NaNO_2$ solution (0.3 ml) was added to each volumetric flask; after 5 min, 10% $AlCl_3$ (w/w) was added; and at 6 min, 1.0 M NaOH (2 ml) was added. Absorbance of the reaction mixture was read at 430 nm.

2.10.4. Estimation of root and leaf lysine content

The lysine content in root and leaf of radish plants was estimated spectrophotometrically following the method of Galicia et al. (2009) with some modifications.

2.10.5. Estimation of root protein, fiber and carbohydrate contents

Protein content (N x 6.25) in dry radish roots was determined according to the AOAC (1990) method 954.01 using Kjeldhal laboratory method. The fiber content was estimated according to method 5983 (ISO 1977). However, total carbohydrates in the radish root were extracted and estimated following the method of Prud'homme et al. (1992).

2.11. Estimation of mineral nutrients in leaf and root

The oven dried plant material (0.1 g of leaf or root) was taken in digestion flasks. A 5 ml of conc. H_2SO_4 was carefully added to each flask (Wolf, 1982) and incubated overnight at room temperature. Afterwards, 0.5 ml H_2O_2 (35%) was poured and the flasks were then placed over a hot plate at 100 °C that was gradually raised to 380 °C until no fumes were produced. The digestion flasks were then removed from the hot plate and allowed to cool. After addition of 0.5 ml of H_2O_2, flasks were again placed on a hot plate (380 °C). This step was repeated until the digestion mixture became transparent. The mixtures were diluted up to 50 ml in volumetric flasks, filtered and stored at 4 °C till further elemental analysis. The content of calcium (Ca), potassium (K) and sodium (Na) were estimated using flame photometer while that of Zinc (Zn), magnesium (Mg) and iron (Fe), were assessed by atomic absorption spectrophotometer (Hitachi. Model 7JO-8024, Tokyo, Japan) using flame spectrophotometry. The phosphorus (P) content of the digested plant material was estimated spectrophotometrically, following the method of Ali and Ashraf (2011) using Barton's reagent. In order to minimize the matrix affect during plant metal analysis, standard reference materials (SRM) and standard solutions were used.

2.12. Statistical analysis

Statistical analysis of data was performed with analysis of variance (ANOVA) by using a statistical program Co-Stat version 6.2, Cohorts Software, 2003, Monterey, CA, USA. Tukey's test (HSD-test) was used for detection of differences among the treatment means ($p \leq .05$). Data was normalized by logarithmic or inverse transformations where necessary prior to the ANOVA.

3. Results

Present study was performed to find out the effects of pre-sowing seed treatment with different regimes of Zn-Lys on seed germination attributes, growth parameters, photosynthetic pigment content, nutrient uptake, varying yield attributes (final root storage) and root nutritional quality at adult stage under water deficit conditions. Data presented in Table 2 shows that PEG-induced water stress imposed significant adverse effects on different studied germination attributes. An increase in mean emergence time (MET) and days to 50% emergence (E_{50}), while a decrease in co-efficient of uniformity of emergence (CUE), germination index (GI), germination percentage (G%) and germination energy (GE) was recorded under PEG-induced water stress. Pre-sowing seed treatment with different regimes of Zn-Lys found effective in reducing the adverse effects of water stress on different germination attributes. More amelioration on different seed germination attributes was recorded due to pre-sowing seed treatment with 3.0 and 4.5 mg kg^{-1} regime of Zn-Lys.

Drought stress caused significant reduction in the leaf as well as root fresh and dry weights, while the Zn-Lys seed treatments found effective in improving the leaf and root fresh and dry biomass under water deficient conditions and comparatively maximum increase was found in plants raised from seeds

Table 2
Seed germination attributes of radish (*Raphanus sativus* L.) influenced by pre-sowing seed treatment with different concentration of Zn-Lys grown in PEG-induced water stress and non-stressed conditions ($n = 3$; mean ± SE).

Zn-lys (ppm)	MET (days)		CUE		GI		G%		E_{50} (days)		GE	
	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
0	3.51 ± 0.03^{a}	3.94 ± 0.02^{a}	0.38 ± 0.006^{d}	0.25 ± 0.001e	8.39 ± 0.51^{d}	5.67 ± 0.17^{b}	90 ± 1.5^{c}	76 ± 1.0^{b}	1.47 ± 0.05^{a}	1.87 ± 0.06^{a}	0.90 ± 0.10^{c}	0.75 ± 0.06^{d}
1.5	2.90 ± 0.02^{b}	3.94 ± 0.03^{a}	0.41 ± 0.002^{c}	0.28 ± 0.002^{d}	10.27 ± 0.62^{c}	7.00 ± 0.18^{a}	95 ± 1.0^{b}	76 ± 1.5^{b}	1.37 ± 0.06$_{b}$	1.75 ± 0.07^{b}	0.95 ± 0.07^{b}	0.80 ± 0.05^{c}
3.0	2.80 ± 0.02^{b}	3.25 ± 0.02^{d}	0.44 ± 0.009^{a}	0.35 ± 0.001^{a}	12.26 ± 0.43^{a}	7.86 ± 0.23^{a}	95 ± 0.5^{b}	83 ± 2.0^{a}	1.23 ± 0.10^{c}	1.61 ± 0.03^{c}	1.00 ± 0.06^{a}	0.85 ± 0.04^{b}
4.5	2.75 ± 0.01^{e}	3.43 ± 0.01^{c}	0.43 ± 0.006^{b}	0.34 ± 0.002^{b}	11.23 ± 0.38^{bc}	7.80 ± 0.12^{a}	96 ± 1.0^{a}	83 ± 2.0^{a}	1.26 ± 0.07^{c}	1.61 ± 0.05^{c}	1.00 ± 0.10^{a}	0.90 ± 0.06^{a}
6.0	2.86 ± 0.02^{c}	3.63 ± 0.02^{b}	0.38 ± 0.006^{d}	0.31 ± 0.003^{c}	11.21 ± 0.47^{bc}	7.25 ± 0.15^{a}	90 ± 1.0^{c}	75 ± 1.5^{c}	1.28 ± 0.08^{c}	1.71 ± 0.04^{b}	0.95 ± 0.08^{b}	0.85 ± 0.05^{b}
LSD 5%	0.04		0.01		0.95		0.54		0.06		0.02	

Values in columns having similar alphabets in superscript do not differ significantly.
NS = non-stressed; WS = water stressed; MET = mean emergence time; CUE = coefficient of uniformity of emergence; GI = germination index; G% = germination percentage; E50 = Time to 50% seed germination; GE = germination energy.

Table 3
Growth attributes of radish (*Raphanus sativus* L.) plants grown from seeds pre-treated with different regimes of Zn-Lys under water stressed and non-stressed conditions ($n = 3$; mean ± SE).

Zn-lys (ppm)	Leaf fresh biomass (g/plant)		Root fresh biomass (g/plant)		Leaf dry weight (g/plant)		Root dry biomass (g/plant)		Root length (cm)		Plant height (cm)	
	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
0	8.03 ± 0.32 ^{b}	5.73 ± 0.17 ^{b}	0.70 ± 0.10 ^{b}	0.50 ± 0.03 ^{b}	0.65 ± 0.05 ^{b}	0.43 ± 0.03 ^{b}	0.08 ± 0.01^{b}	0.043 ± 0.01^{b}	13.8 ± 0.43 ^{b}	10.9 ± 0.64 ^{b}	19.0 ± 2.0 ^{b}	15.0 ± 1.0 ^{c}
1.5	9.76 ± 0.44 ^{a}	6.20 ± 0.47 ^{ab}	0.78 ± 0.08 ^{ab}	0.55 ± 0.06 ^{b}	0.74 ± 0.06 ^{a}	0.57 ± 0.03^{a}	0.11 ± 0.01^{a}	0.043 ± 0.01^{b}	15.0 ± 0.87 ^{b}	11.2 ± 0.75 ^{a}	19.9 ± 1.0 ^{a}	17.0 ± 1.0 ^{b}
3.0	9.47 ± 0.55 ^{a}	7.17 ± 0.45 ^{a}	0.83 ± 0.03 ^{a}	0.57 ± 0.04 ^{b}	0.71 ± 0.07 ^{b}	0.62 ± 0.04^{a}	0.12 ± 0.01^{a}	0.067 ± 0.01^{a}	14.3 ± 0.22 ^{b}	11.4 ± 0.37 ^{a}	20.1 ± 1.0 ^{a}	17.2 ± 0.7 ^{b}
4.5	10.33 ± 0.88 ^{a}	7.47 ± 0.59 ^{a}	0.84 ± 0.06 ^{a}	0.68 ± 0.05 ^{a}	0.75 ± 0.05 ^{ab}	0.64 ± 0.06^{a}	0.12 ± 0.01^{a}	0.070 ± 0.01^{a}	18.8 ± 0.24^{a}	11.3 ± 0.43 ^{a}	20.6 ± 0.9 ^{a}	17.6 ± 0.3 ^{b}
6.0	9.67 ± 1.00 ^{a}	7.00 ± 0.58 ^{ab}	0.88 ± 0.05 ^{a}	0.63 ± 0.05 ^{a}	0.82 ± 0.05 ^{a}	0.58 ± 0.03^{a}	0.12 ± 0.01^{a}	0.057 ± 0.01^{ab}	18.8 ± 0.46^{a}	11.5 ± 0.51 ^{a}	20.3 ± 0.9 ^{a}	20.2 ± 0.7 ^{a}
LSD 5%	1.35		0.11		0.10		0.016		1.25		1.23	

Values in columns having similar alphabets in superscript do not differ significantly.
NS = non-stressed; WS = water stressed.

Table 4
Leaf carotenoids, chlorophylls, lysine and total soluble protein content of radish (*Raphanus sativus* L.) plants grown from seeds pre-treated with different regimes of Zn-Lys under water stressed and non-stressed conditions ($n = 3$; mean ± SE).

Zn-lys (ppm)	Leaf carotenoids (mg/g FW)		Chl. *a* (mg/g FW)		Chl. *b* (mg/g FW)		Total Chl. (mg/g FW)		Chl. *a*/*b*		Leaf Lys (mg/kg)		Leaf total soluble protein (mg/kg FW)	
	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
0	1.31 ± 0.14 ^{b}	1.16 ± 0.14 ^{d}	2.70 ± 0.21 ^{c}	1.91 ± 0.12 ^{b}	1.06 ± 0.13 ^{b}	0.66 ± 0.06 ^{b}	3.15 ± 0.13 ^{c}	2.44 ± 0.09 ^{c}	2.58 ± 0.13 ^{a}	2.89 ± 0.09 ^{a}	122 ± 3.7^{b}	98 ± 3.4^{b}	1666 ± 68^{c}	1433 ± 36^{c}
1.5	0.98 ± 0.23 ^{c}	1.38 ± 0.13 ^{ab}	2.96 ± 0.10 ^{abc}	2.17 ± 0.10 ^{ab}	1.25 ± 0.10 ^{a}	1.02 ± 0.06 ^{a}	3.54 ± 0.15 ^{ab}	2.65 ± 0.10 ^{bc}	2.39 ± 0.11 ^{ab}	2.14 ± 0.10^{b}	126 ± 1.8^{ab}	105 ± 2.9^{b}	1700 ± 58^{bc}	1500 ± 58^{bc}
3.0	1.21 ± 0.20 ^{b}	1.31 ± 0.15 ^{bc}	2.76 ± 0.10 ^{bc}	2.40 ± 0.12 ^{a}	1.23 ± 0.10 ^{a}	1.06 ± 0.08 ^{a}	3.47 ± 0.10 ^{abc}	2.99 ± 0.11 ^{ab}	2.27 ± 0.20 ^{b}	2.31 ± 0.12^{b}	128 ± 3.5^{ab}	128 ± 3.2^{a}	1800 ± 58^{ab}	1566 ± 33^{b}
4.5	1.44 ± 0.20 ^{a}	1.21 ± 0.10 ^{cd}	3.23 ± 0.11 ^{a}	2.40 ± 0.11 ^{a}	1.37 ± 0.10 ^{a}	1.16 ± 0.03 ^{a}	3.37 ± 0.13 ^{bc}	3.19 ± 0.07 ^{a}	2.37 ± 0.10 ^{ab}	2.08 ± 0.09^{b}	131 ± 2.1^{a}	123 ± 4.0^{a}	1866 ± 33^{a}	1600 ± 48^{a}
6.0	1.46 ± 0.15 ^{a}	1.51 ± 0.11 ^{a}	3.04 ± 0.12 ^{ab}	2.30 ± 0.10 ^{a}	1.21 ± 0.10 ^{a}	1.08 ± 0.05 ^{a}	3.73 ± 0.12 ^{a}	3.16 ± 0.16 ^{a}	2.51 ± 0.12 ^{ab}	2.14 ± 0.05^{b}	133 ± 3.7^{a}	123 ± 3.3^{a}	1800 ± 58^{ab}	1566 ± 37^{b}
LSD 5%	0.14		0.32		0.18		0.33		0.30		7.80		105	

Values in columns having similar alphabets in superscript do not differ significantly.
NS = non-stressed; WS = water stressed.

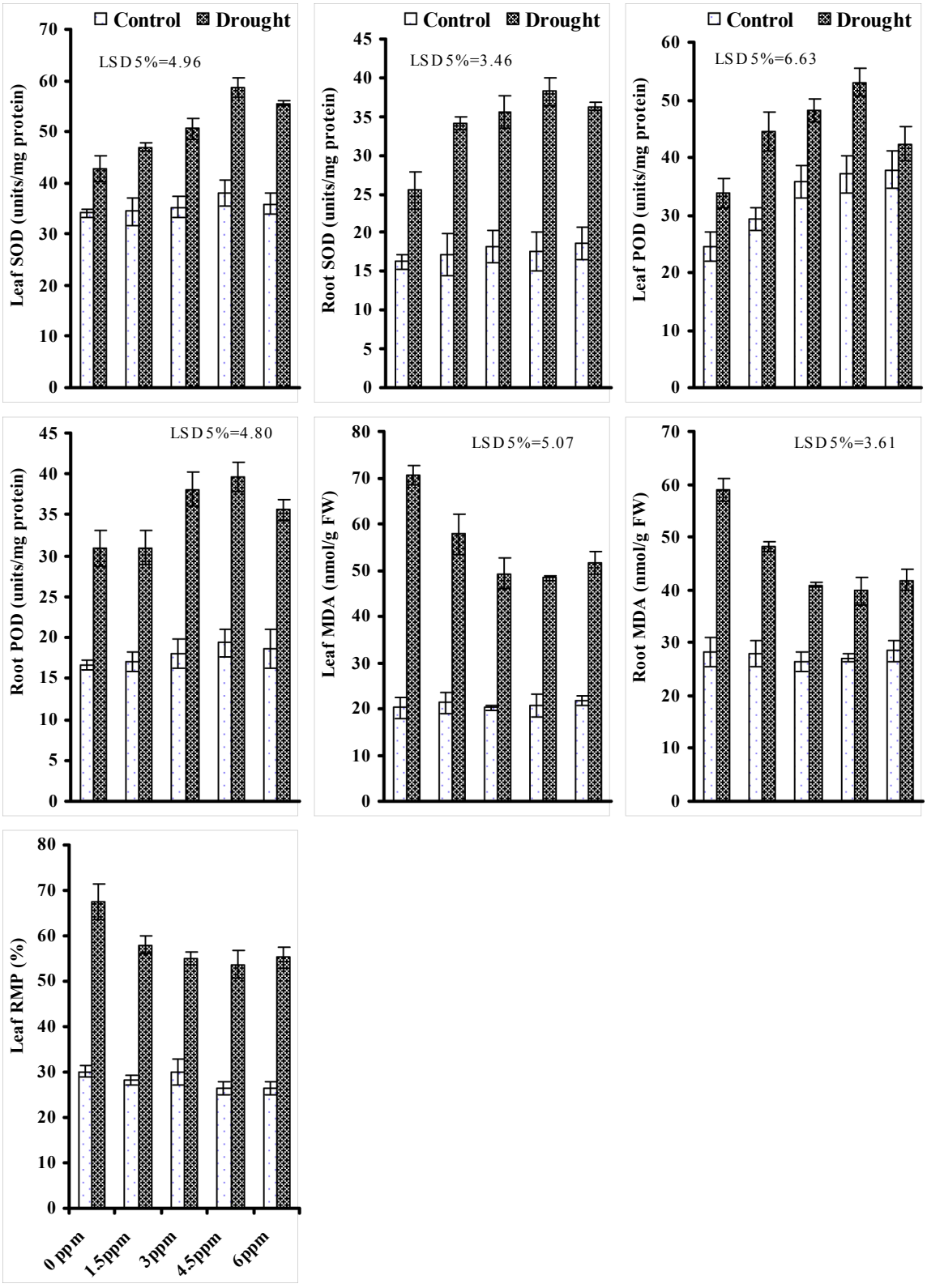

Fig. 1. Activities of antioxidant enzymes, MDA accumulation in root and shoot and leaf relative membrane stability radish (*Raphanus sativus* L.) plants effected due to pre-sowing seed treatment with different regimes of Zn-Lys under water stressed and non-stressed conditions (mean ± SE; $n = 3$).

observed at 4.5 mg kg^{-1} Zn-Lys treatment. Under non-stressed condition, the leaf and root fresh and dry biomass also increased together due to pre-sowing seed treatment with Zn-Lys and comparatively but non-significantly the maximum increase was observed in plants raised from seeds pre-treated with 4.5 mg kg^{-1} of Zn-Lys (Table 3).

A significant decrease in plant height and root length was found due to water stress of radish plants. The priming of seeds with Zn-

Table 5
Means of Na, K, Ca and Zn of radish (*Raphanus sativus* L.) plants grown from seeds pre-treated with different regimes of Zn-Lys under water stressed and non-stressed conditions ($n = 3$; mean ± SE).

Zn-lys (ppm)	Leaf Na^+ (mg/g DW)		Leaf K^+ (mg/g DW)		Leaf Ca^{2+} (mg/g DW)		Leaf Zn^{2+} (mg/g DW)	
	NS	WS	NS	WS	NS	WS	NS	WS
0	0.33 ± 0.05^{a}	0.28 ± 0.04^{a}	26.27 ± 2.33^{c}	15.97 ± 0.50 ^{c}	3.07 ± 0.23 ^{c}	2.30 ± 0.11 ^{c}	13.07 ± 0.23 ^{c}	7.83 ± 0.44 ^{a}
1.5	0.33 ± 0.07^{a}	0.26 ± 0.03^{a}	29.57 ± 1.69^{bc}	19.30 ± 0.75 ^{bc}	3.50 ± 0.15 ^{ab}	2.93 ± 0.33 ^{ab}	14.90 ± 0.32 ^{b}	8.50 ± 0.29 ^{a}
3.0	0.34 ± 0.04^{a}	0.27 ± 0.04^{a}	38.00 ± 2.17^{a}	19.77 ± 1.00 ^{abc}	3.77 ± 0.17 ^{a}	2.67 ± 0.18 ^{b}	15.43 ± 0.75 ^{b}	9.47 ± 0.29 ^{b}
4.5	0.31 ± 0.05^{a}	0.28 ± 0.03^{a}	38.33 ± 1.84 ^{c}	23.97 ± 1.30 ^{a}	3.65 ± 0.31 ^{a}	2.89 ± 0.14 ^{ab}	16.95 ± 0.07 ^{a}	10.83 ± 0.44 ^{a}
6.0	0.34 ± 0.03^{a}	0.27 ± 0.04^{a}	31.93 ± 1.50^{b}	23.13 ± 1.10 ^{ab}	3.27 ± 0.24 ^{bc}	3.04 ± 0.20 ^{a}	17.60 ± 0.56 ^{a}	11.50 ± 0.29 ^{a}
LSD 5%	0.30		4.42		0.36		0.85	

Values in columns having similar alphabets in superscript do not differ significantly.
NS = non-stressed; WS = water stressed.

Lys recovered the plants from stress and 6 mg kg^{-1} concentration proved better than all other applied levels (i.e. 1.5, 3 and 4.5 mg kg^{-1}), which produced almost similar recovery effects. The priming of seeds with different regimes of Zn-Lys under non-stressed conditions also resulted in an increase in plant height and root length (more at 4.5 and 6 mg kg^{-1}) of radish plants (Table 3).

3.1. Leaf photosynthetic pigments

Imposition of drought stress significantly reduced the leaf Chl. *a*, Chl. *b*, total Chl. and leaf carotenoid contents of radish plants and comparatively, higher reduction was observed for Chl. *b* than the Chl. *a*. The Zn-Lys seed priming significantly enhanced the leaf Chl. *a*, *b*, and total Chl. as well as leaf carotenoid contents, under stressed and non-stressed conditions, and to the maximum increase was found in plants raised from seeds pre-treated with 4.5 and 6.0 mg kg^{-1} regimes of Zn-Lys but in case of Chl. b this increase among treatments was not significant (Table 4). However, the leaf chlorophyll *a*/*b* ratio increased significantly under water deficit conditions. Pre-sowing seed treatment with different regimes of Zn-Lys significantly decreased the leaf chlorophyll *a*/*b* of radish plants but the decreasing trend was different under stressed and non-stressed conditions. Under water deficit conditions, 4.5 mg kg^{-1} and in non-stressed conditions 3 mg kg^{-1} of Zn-Lys was the most effective dose but this effect was not significant among treatments both under stressed and non-stressed conditions (Table 4).

3.1.1. Leaf lysine and total soluble protein contents

Leaf lysine and total soluble protein contents decreased significantly due to imposition of drought stress. A significant increase was recorded in leaf lysine and total soluble contents in radish plants raised from seeds pre-treated with different regimes of Zn-Lys. The maximum increase in leaf total soluble proteins both under stressed and non-stressed conditions was found in plants raised from seeds pre-treated with 4.5 mg kg^{-1} of Zn-Lys as compared with other regimes; while in case of leaf lysine this increase was maximum in plants raised from seeds pre-treated with 6.0 mg kg^{-1} of Zn-Lys (Table 4).

3.1.2. Activities of antioxidant enzymes and lipid peroxidation in leaf and root

The SOD and POD activity in leaf and root of radish plants increased significantly due to imposition of drought stress. A further increase in SOD and POD activity both in leaf and root was found in plants raised from seeds pre-treated with different regimes of Zn-Lys and the maximum increase was found in plants raised from seeds pre-treated with 4.5 mg kg^{-1} of Zn-Lys as compared with other regimes (Fig. 1). Leaf and root MDA contents and leaf relative membrane permeability also increased significantly due to imposition of drought stress. However, a significant reduction in leaf and root MDA content as well as leaf relative membrane permeability was found in plants raised from seeds pre-treated with different Zn-Lys regimes and the maximum reductions in leaf and root MDA contents and leaf relative membrane permeability were noted in plants raised from seeds pre-treated with 4.5 mg kg^{-1} of Zn-Lys as compared with other regimes (Fig. 1).

3.1.3. Leaf nutrient content

Drought stress significantly decreased the leaf Na, K, Ca and Zn content. A significant increase in these nutrients in leaves was found in plants raised from seeds pre-treated with different regimes of Zn-Lys except to that of leaf Na that remained unaffected due to Zn-Lys pre-sowing seed treatment both under stressed and non-stressed conditions. In relation with leaf K content, the maximum increase was found in plants raised from seeds pre-treated with 4.5 mg kg^{-1} of Zn-Lys. In case of leaf Ca content, this increase was maximum in plants raised from seeds pre-treated with 6 mg kg^{-1} of Zn-Lys under water stress, while in non-stressed plants this increase was maximum in plants raised from seeds pre-treated with 3 mg kg^{-1} of Zn-Lys. However, in case of leaf Zn the maximum increase was found due to 6 mg kg^{-1} of Zn-Lys pre-sowing seed treatment both under water stressed and non-stressed conditions (Table 5).

3.1.4. Root yield attributes and nutritional quality

Data presented in Fig. 2 shows that imposition of drought stress significantly reduced the root yield, root final length and diameter. Pre-sowing seed treatment with different regimes of Zn-Lys was found effective in reducing the adverse effects of drought on these yield attributes but the most effective dose was parameter specific. In case of root yield, 4.5 mg kg^{-1} of Zn-Lys was most effective under stress and non-stress conditions. However, in case of root length root diameter under water stress 1.5 and 3 mg kg^{-1} and 3 mg kg^{-1} of Zn-Lys respectively, were the most effective while under no-stressed conditions 3 and 4.5 mg kg^{-1} were the effective concentrations for both attributes.

Data presented in Fig. 3 for varying root nutritional quality attributes shows that root protein and fiber contents decreased, while that of carbohydrate increased significantly due to drought stress. However, a significant increase in these attributes was found in plants grown from seeds pre-treated with different regimes of Zn-Lys both under water stressed and non-stressed conditions. Among different Zn-Lys levels, 3 and 4.5 mg kg^{-1} were comparatively most effective as compared with other regimes both under stressed and non-stressed conditions.

Imposition of drought stress significantly increased the root flavonoid, carotenoids and lysine contents of radish plants while that of root ascorbic acid content decreased. Pre-sowing seed

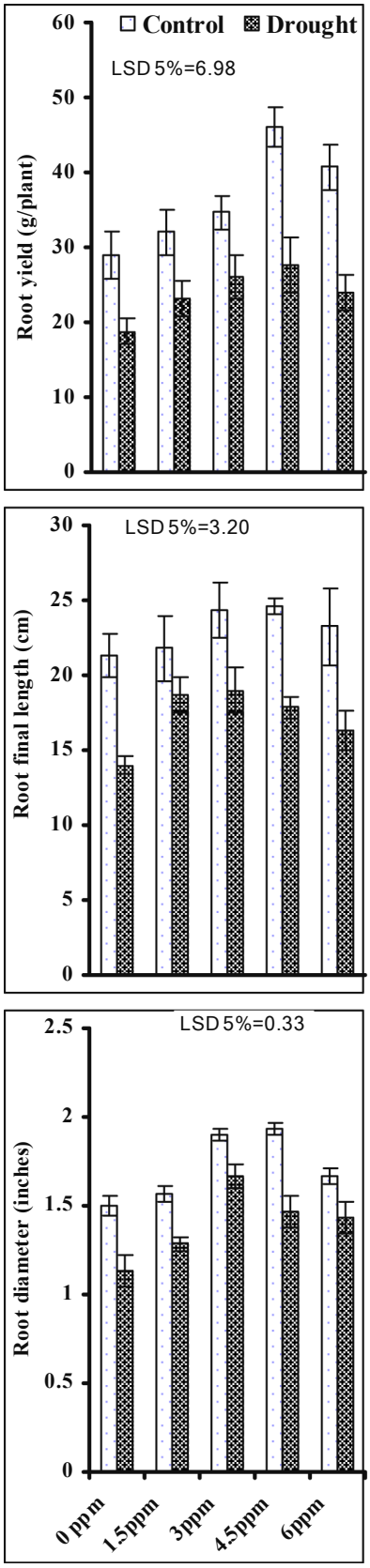

Fig. 2. Root yield attributes of radish plants (*Raphanus sativus* L.) effected due to pre-sowing seed treatment with different regimes of Zn-Lys under water stressed and non-stressed conditions (mean ± SE; $n = 3$).

treatment with different regimes of Zn-Lys further enhanced the root flavonoid, carotenoids and lysine contents both under stressed and non-stressed conditions and 3 and 4.5 mg kg^{-1} seed treatment was found most effective as compared with other regimes. However, root ascorbic acid content increased significantly in plants raised from seeds pre-treated with different regimes of Zn-lys and the maximum amelioration was found due to 3 mg kg^{-1} Zn-Lys level (Fig. 3).

Data presented in Table 6 for different root minerals nutrients such as Na, K, Ca, Zn. Fe, Mg and P shows that imposition of drought stress significantly reduced them except to that of Na that remain unaffected. Seed priming with different regimes of Zn-Lys found effective in increasing the root mineral content under stressed and non-stressed conditions. Of different Zn-Lys levels, the seed treatment with 4.5 mg kg^{-1} level was found most effective in increasing the root nutrients as compared with other regimes both under stressed and non-stressed conditions.

In view of the PCA analysis presented in Fig. 4, it can be confirmed that there is a strong relations (positive/negative) among different growth and seed germination attributes. From the dendrograms among different growth, biochemical, antioxidant enzymes, lipid peroxidation and nutrient contents as presented in Fig. 5, it can be inferred that the lipid peroxidation and anti-oxidative enzymes are positively correlated and are grouped in a similar class. Strong positive correlation was found among leaf and root MDA content with root SOD, root and leaf POD and LRMP. However, the root and shoot biomass production, photosynthetic pigments, and leaf nutrient contents are grouped in other single class. Leaf Zn and Lys are having strong positive correlations with leaf chlorophylls, fresh and dry biomass and leaf nutrients.

4. Discussion

The most prominent effect of water deficit is on reduced seed germination and seedling emergence that impact crop stand establishment and plant growth. In the current study, PEG-induced water stress imposed significant adverse effects on all studied seed germination attributes (G%, GI, CUE, MET, GE and E_{50}) that might be due to reduced water uptake by seeds, leading to reduced activity of germination enzymes (Muscolo et al., 2014; Ali et al., 2016). A seed's capacity to absorb water plays a significant role in seed germination and early seedling establishment (Arjenaki et al., 2011; Zaynab et al., 2017). An improvement in seed germination attributes and seedling emergence due to Zn-Lys priming might be due to it lowering seed osmotic potential (Patane et al., 2013). As a result of the Zn-Lys priming, seeds absorb more water after sowing, resulting in increased activities of germination enzymes and finally better germination and seedling emergence, as reported by Muscolo et al. (2014) and Ali et al. (2016) in different cultivars of lentil and mash, respectively, under PEG-induced water-stress conditions. Our opinion correlates with the previous studies, revealing that better seed germination and seedling vigor contribute towards obtaining better yields in field grown crops under adverse environmental conditions (Singh et al., 2015). In the present study, seed priming with different Zn-Lys regimes improved seed germination attributes, perhaps due to the role of Lys in improving seed energy status as reported in several studies (Angelovici et al., 2011; Galili and Amir, 2013; Galili et al., 2014), and with a consequent improved germination, seedling emergence, and better crop stand establishment under stressed conditions.

Drought stress exerted a deteriorating impact on leaf biomass of radish plants. It is likely that the adverse effect arose from the incursion of roots into dry soil and root respiratory insufficiency

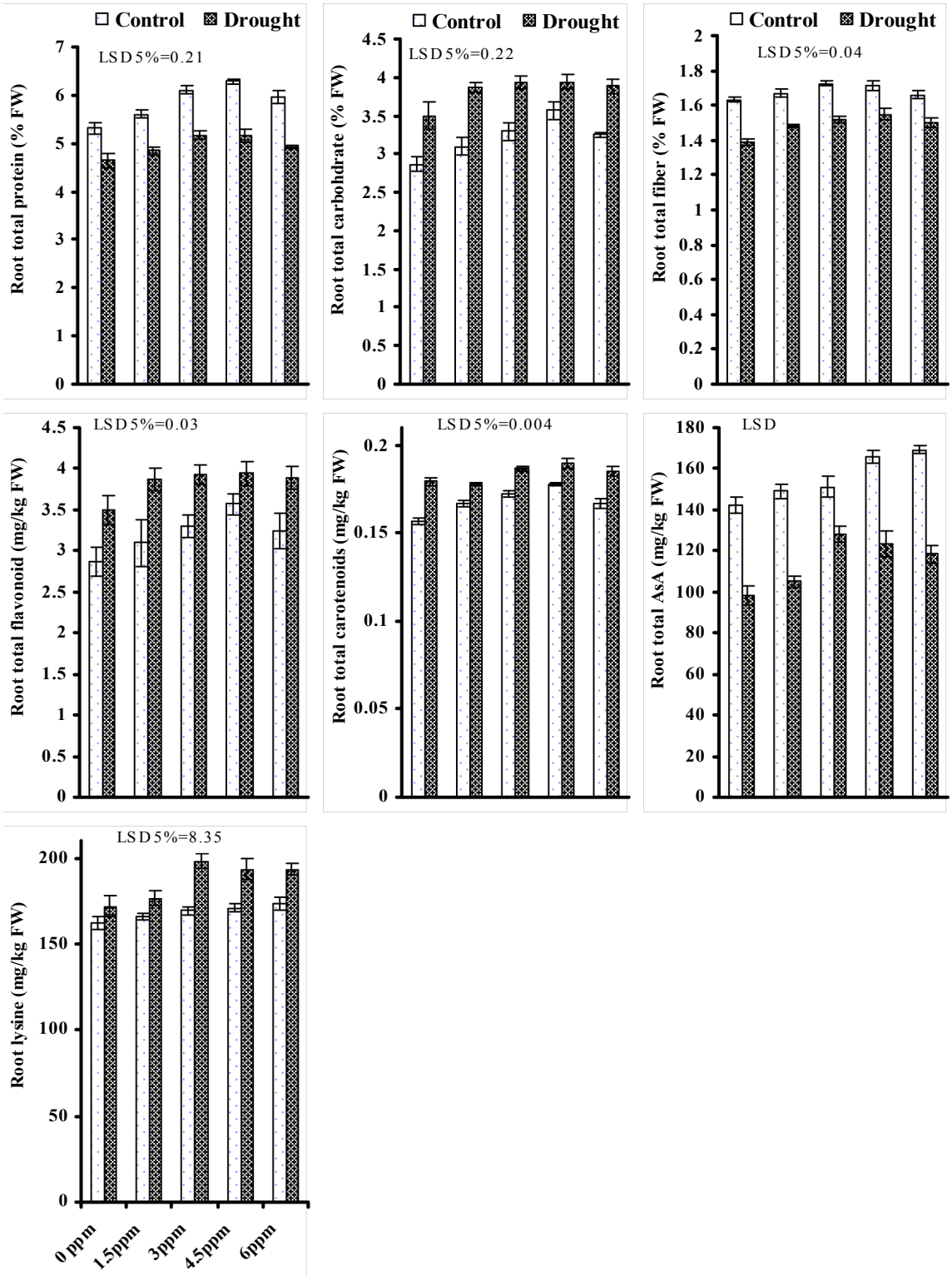

Fig. 3. Root nutritional quality attributes of radish (*Raphanus sativus* L.) plants effected due to pre-sowing seed treatment with different regimes of Zn-Lys under water stressed and non-stressed conditions (mean ± SE; $n = 3$).

(Thomas and Howarth, 2000). A reduction in root and shoot fresh masses indicated disturbed water relations, and it has been previously suggested that an application of Zn-Lys might improve water relations under conditions of stress, thereby establishing an alleviatory role under drought (Hafeez et al., 2013). Increments in root and shoot dry weights after Zn-Lys treatment indicated the improved photosynthetic activity of *R. sativus* plants was due to enhanced synthesis of leaf chlorophyll contents. Fang et al. (2015) found that in strawberry leaves, enhanced Lys acylation in chloroplasts was associated with increased photosynthesis. This can be correlated with the present findings where priming with Zn-Lys enhanced the leaf Lys and chlorophyll contents, that might

Table 6
Mineral composition of the final harvested root of radish (*Raphanus sativus* L.) plants grown from seeds pre-treated with different regimes of Zn-Lys under water stressed and non-stressed conditions ($n = 3$; mean ± SE).

Zn-lys (ppm)	Na^+ (mg/g DW)		K^+ (mg/g DW)		Ca^{2+} (mg/g DW)		Zn (mg/g DW)		Fe (mg/kg DW)		Mg^{2+} (mg/kg DW)		P (mg/kg DW)	
	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS	NS	WS
0	0.39 ± 0.01 ^{a}	0.37 ± 0.03 ^{a}	37.0 ± 1.16 ^{c}	23.7 ± 0.88 ^{c}	6.23 ± 0.17 ^{c}	3.65 ± 0.15 ^{a}	6.23 ± 0.17 ^{d}	3.65 ± 0.15 ^{c}	2.97 ± 0.14 ^{c}	2.30 ± 0.11 ^{b}	100 ± 5.8 ^{b}	80.±2.9 ^{b}	183 ± 3.3 ^{d}	157 ± 4.4 ^{c}
1.5	0.36 ± 0.02 ^{a}	0.36 ± 0.01 ^{a}	40.3 ± 0.88 ^{ab}	24.5 ± 1.01 ^{c}	6.51 ± 0.22 ^{bc}	3.68 ± 0.13 ^{a}	6.85 ± 0.16 ^{c}	3.78 ± 0.06 ^{c}	3.40 ± 0.06 ^{ab}	2.48 ± 0.08 ^{a}	103 ± 9.3 ^{b}	87 ± 4.4 ^{a}	188 ± 4.4 ^{cd}	160 ± 2.9 ^{bc}
3.0	0.35 ± 0.01 ^{b}	0.34 ± 0.01 ^{a}	41.7 ± 2.60 ^{a}	30.0 ± 2.03 ^{ab}	6.79 ± 0.10 ^{ab}	3.70 ± 0.30 ^{a}	7.08 ± 0.21 ^{b}	4.14 ± 0.12 ^{b}	3.50 ± 0.11 ^{a}	2.57 ± 0.13 ^{a}	111 ± 4.3 ^{a}	90 ± 5.0 ^{a}	196 ± 3.3 ^{ab}	167 ± 4.4 ^{ab}
4.5	0.37 ± 0.02 ^{a}	0.36 ± 0.01 ^{a}	42.7 ± 0.88 ^{a}	28.6 ± 2.33 ^{b}	7.05 ± 0.20 ^{a}	3.82 ± 0.25 ^{a}	7.38 ± 0.13 ^{b}	4.35 ± 0.08 ^{a}	3.50 ± 0.11 ^{a}	2.63 ± 0.04 ^{a}	112 ± 1.7 ^{a}	92 ± 6.7 ^{a}	200 ± 5.0 ^{a}	168 ± 3.5 ^{a}
6.0	0.34 ± 0.01 ^{b}	0.37 ± 0.03 ^{a}	37.7 ± 0.88 ^{bc}	31.8 ± 0.82 ^{a}	6.95 ± 0.14 ^{a}	3.74 ± 0.15 ^{a}	7.93 ± 0.12 ^{a}	4.58 ± 0.12 ^{a}	3.28 ± 0.07 ^{b}	2.52 ± 0.09 ^{a}	103 ± 3.7 ^{b}	87 ± 4.4 ^{a}	192 ± 1.7 ^{bc}	164 ± 2.1 ^{abc}
LSD 5%	0.038		3.13		0.40		0.29		0.21		8.64		7.62	

increased the photosynthesis and assimilation which resulted in enhanced plant growth and root yield under stressed and non-stressed conditions. Furthermore, it is reported by Araújo et al. (2010) that under conditions of limited photosynthesis, Lys is directly catabolized in the TCA cycle to maintain the required energy status. Increased biomass, therefore, offers new binding sites for nutrient cations (Javed, 2011; Javed et al., 2014), which, in turn, result in improved plant growth. It is also likely that the production of respiratory intermediates like Acetyl CO-A and glutamate increased in Zn-Lys primed seeds that have positive roles in seed respiration. Since respiration remains at efficient levels even under drought conditions, *R. sativus* growth enhancement was observed in the present study. In parallel with Zn positive effects on growth, Lys also likely takes part in enhancing growth because during Lys degradation, sachropine dehydrogenase yields α-aminoadipic-δ-semialdehyde, an intermediate involved in gene regulation for nitrogen metabolism (Arruda et al., 2000). So, it can be inferred that degradation of Zn-Lys yielded α-aminoadipic-δ-semialdehyde and glutamate, which, in turn, contributed to *R. sativus* growth under normal as well as water-deficit conditions. Zn-Lys triggered *R. sativus* to boost root length under water scarcity; this might be due to enhanced growth of shoot system that resulted in an incremented synthesis of photo-assimilates and finally a speedy shift in source sink partitioning by transferring more photosynthates towards the roots (Wei et al., 2007).

Leaf photosynthetic pigments such as Chl. *a*, *b*, *a*/*b*, total Chl. and carotenoids decreased when plants faced drought conditions. It has been found that drought stress decreases plant photosynthetic rate due to differences between light capture and its utilization (Foyer and Noctor, 2000). Zn-Lys priming produced a significant increase in leaf chlorophyll and carotenoid content, which, in turn, contributed to the enhancement of *R. sativus* growth due to improved photosynthetic activity under water stress. Zafari and Ebadi (2016) stated that, in canola, an increase or decrease in leaf carotenoids occurs with an increase or decrease in Lys content, respectively. Lysine is therefore, likely, to be helpful in its tolerance of stress. Similar findings were noted in the present study where Zn-Lys treatment increased leaf Lys content and thus leaf carotenoid content.

Excessive ROS production causes damage to macromolecules including cellular membrane lipids, resulting in leaky membranes. Due to their unsaturated nature, membrane lipids are the foremost target of ROS resulting in MDA accumulation; this is the parameter used to measure the extent of oxidative stress damage. In our study, seed treatment with Zn-Lys significantly improved the relative membrane permeability of treated radish plants, which boosted their survival rate under water deficiency. These results can be correlated with the findings that Zn prevents the peroxidation of membrane components (Cakmak, 2000) and acts to stabilize cell membranes through interaction with phospholipids and sulfydril groups of membrane proteins (Marschner, 2011). Hence, drought stress resulted in a significant increase in MDA production in both roots and shoots, confirming increased ROS production as well as membrane lipid peroxidation in *R. sativus* as noted by earlier studies (Joseph et al., 2011; Lee et al., 2012). Increased MDA content at a cellular level, resulting in more oxidative injury, is linked with a disruption of ionic balance (Hernández and Almansa, 2002; Azooz et al., 2009). It has been previously noted that lipid peroxidation and ionic leakage induced by drought correspond with reduced leaf water potential (Apel and Hirt, 2004) and a consequent reduction in growth and yield. Plant anti-oxidative systems usually provide a defense mechanism against the deleterious actions of ROS (Ali and Ashraf, 2011; Zafar et al., 2016; Noman et al., 2017a). In this regard, SOD and POD, being enzymatic anti-oxidative components, play an important role in the detoxification process (Noman et al., 2017b).

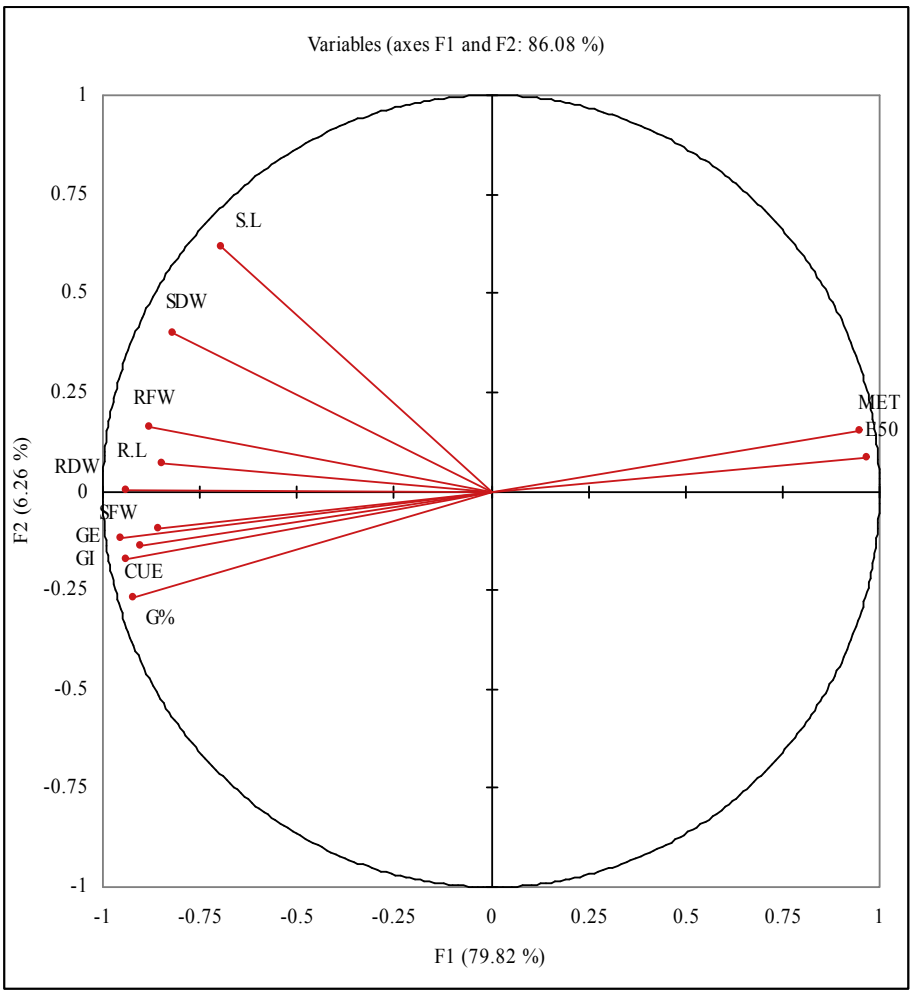

Fig. 4. Principle component analysis of seed germination and growth attributes of radish (*Raphanus sativus* L.) plants.

Zn-Lys seed treatment significantly functions in enhancing the activities of antioxidant enzymes and the content of non-enzymatic antioxidants such as leaf and root carotenoids. Our results suggested that Zn-Lys priming protected lipid peroxidation and contributed to enzymatic and non-enzymatic antioxidant defense mechanisms in *R. sativus*.

The reported increase in SOD and POD activity after Zn-Lys priming is likely to be an adaptive trait of *R. sativus* against drought (Noman et al., 2015; Ali et al., 2016; Noman and Aqeel, 2017). *R. sativus* plants obtained from Zn-Lys primed seeds exhibited higher SOD activity as compared to plants raised from non-primed and hydro-primed seeds. Zinc acts as a co-factor for SOD and it does not interfere directly with oxidant species. In order to perform its antioxidant function, Zn binds to thiol group of protein (i.e. metallothioneins and Zn finger TFs) leading to their stabilization (Powell, 2000). So, in the present study, Zn might have played a significant role by acting as a co-factor in stabilizing proteins. Under drought stress, POD activity was also increased in *R. sativus*. It has been found that hydrolytic POD activity increases under drought due to protein degradation and increased soluble protein cognitive content (Ashraf, 2010). Furthermore, POD activity exhibits a steady increase in *R. sativus* plants grown from seeds pretreated with Zn-Lys, both under normal irrigation and drought conditions.

Uptake of the essential mineral nutrients plays significant role in coping the stressful environmental conditions especially the water stress. In the present study, a significant reduction was found in the uptake of all studied mineral nutrients under drought conditions. Since the mineral elements of plants play an important role in plant resistance to drought stress (da Silva et al., 2011), therefore, the management of nutrients is essential to create tolerance in crop plants under drought stress (Ali et al., 2016). It was found that mineral elements not only contribute in cellular osmotic adjustment and redox potential but also minimize the adverse effect of ROS by boosting up the activities of the antioxidant enzymes like POD, SOD and CAT (Waraich et al., 2011; Ali et al., 2016). Zn-Lys presowing seed treatment was found effective in reducing the adverse effects of drought on the uptake of mineral nutrients such as K, Ca, and Na in leaves and roots as well as the accumulation of Fe, Mg, P, and Zn. This increment in nutrient uptake due to Zn-Lys pre-sowing seed treatment might be the function of various physio-biochemical factors as well as root structural properties (Egilla et al., 2001). An increase in root length/growth is one such factor under Zn-Lys seed priming, as reported in the present study, resulting in a greater uptake of soil available nutrients. Furthermore, it was found that Zn nutrition play important role in nutrient uptake under water deficit conditions due to its role in stomatal conductance by effecting plant water relations. The role of lysine catabolism during the degradation of Zn-Lys to glutamate or proline might be the other factor, as reported by (Zafari and Ebadi, 2016) in canola. It shows that lysine catabolism lowers cellular osmotic potential and causes a boost in uptake of nutrients along

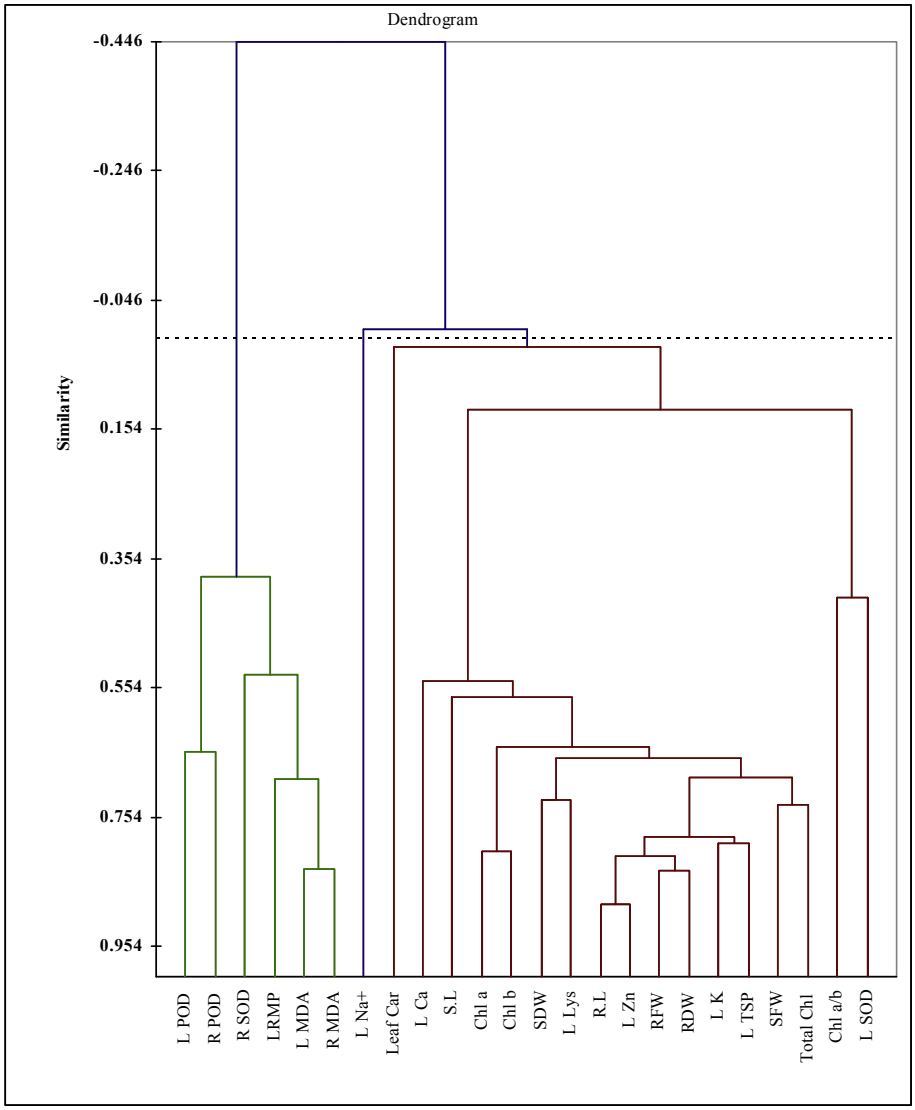

Fig. 5. Dendrograms among different growth, photosynthetic pigments, antioxidative enzymes activities and leaf nutrients.

with water, with consequent increased growth of the shoot system coupled with high assimilation of photosynthates. This resulted in increased root yield and nutritional quality in terms of root proteins, carbohydrates, fibers, flavonoids, carotenoids, and AsA, as well as root Lys content. The increased root and/or nutritional status was found to be effective in reducing the adverse effects of drought on *R.sativus* plants. Furthermore, Zn-Lys priming enhanced the Zn and Lys content of roots and leaves which positively relates with the studied minerals and other compounds like flavonoids, AsA, proteins, fiber, carbohydrates, in root as well as in leaf. This improvement might be due to the role of Zn which has shown positive effects on studied minerals by maintaining cell membrane stability/integrity, linked with NADPH-dependent O_2 production. El Fouly et al. (2010) reported that Zn has a role in nutritional preconditioning in *Vicia faba*. Similarly, it has been found that the activity of lysine ketoglutaric acid reductase during lysine catabolism is associated with Ca uptake (Galili et al., 2001).

5. Conclusions

We envisioned that Zn-Lys induced its effects through the modulation of seed germination attributes, photosynthetic activity and anti-oxidative defense mechanisms. Moreover, Zn-Lys also exerted a synergistic effect on the uptake of Zn, Na, K, and Ca in order for *R. sativus* to achieve optimum nutrient levels by preventing drought-induced lipid peroxidation of membranes. Increments in root yield and nutritional quality by maintaining more accumulation/synthesis of photosynthates (protein, carbohydrates, fiber, flavonoids, carotenoids, lysine, and ascorbic acid along with increased levels of mineral nutrients (Na, K, Ca, Mg, Fe, P), is an additional benefit of Zn-Lys priming. Further exploration of the mechanisms responsible for Zn-Lys induced growth modulation in plants may provide an effective approach for optimizing strategies to produce bio-fortified food and for minimizing environmental pollution in arid and semi-arid regions. However, future studies, using modeling and experimental approaches, should be conducted to draw parallels among Zn-Lys priming, arable crops/plants, elemental mobility patterns in drought-stressed rhizospheres, plant growth, and environmental restoration.

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